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Short Communication

Determination of phenylbutazone decomposition products by mass fragmentography and derivative UV-spectrophotometry

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Introduction

Phenylbutazone (I) is a pharmaceutical substance widely used as an anti-arthritic and analgesic agent.

The major degradation products of phenylbutazone are shown in Scheme 1: 4-butyl-4-hydroxy-1,2-diphenylpyrazolidine-3,5-dione (II), butylpropanedioic acid mono(1,2-diphenylhydrazide) (III), butylhydroxypropanedioic acid mono(1,2-diphenylhydrazide) (IV) and hexanoic acid 1,2-diphenylhydrazide (V).

Among these decomposition substances, II is a potential immunogenic contaminant, possibly involved in allergic reactions [1].

Previously, the quantitation of II has been measured by TLC [2, 3], GC [4] and HPLC [5–7]. Compound III has been determined by TLC [2, 3], GC [4, 8], UV-spectrophotometry [9] and HPLC [7]; IV by TLC [2, 3], GC [4, 10] and HPLC [5, 7]. The determination of V has been carried out by TLC [2], GC [4] and HPLC [7].

The present paper describes the determination of these decomposition products in bulk material by mass fragmentography and derivative UV-spectrophotometry.

Experimental

Reagents and chemicals

Ethanol was of spectroscopic reagent grade. Phenylbutazone was purified by several recrystallizations of the commercial product from ethanol–water. Compounds II and IV were prepared according to Giraldi and Tosolini [11]; compound III according to Schmid

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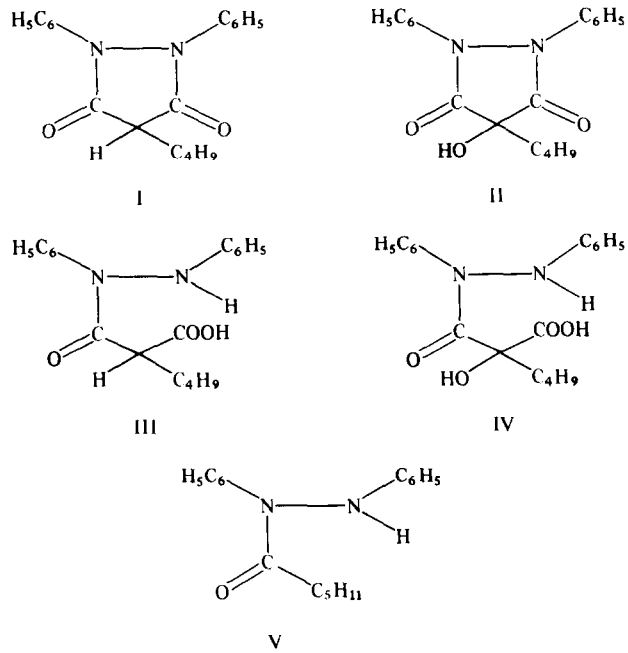
Scheme 1



[9] and compound V according to Hallmann and co-workers [12]. Phenazone (2,3-dimethyl-1-phenyl-3-pyrazolin-5-one) was of analytical reagent grade.

Apparatus and conditions

Mass spectrometric analyses were recorded on a low-resolution mass spectrometer with a data system (LKB Model 2091/2130). The ionization energy and accelerating voltage were 20 eV and 3500 V, respectively. The ion source temperature was 250°C. The probe temperature was increased from 20 to 200°C in 5 min.

The UV-spectra were obtained with a Perkin–Elmer Model 320 ultraviolet-visible spectrophotometer. Zero order spectra: scan speed, 120 nm min^{-1}; spectral slit-width, 2 nm. Derivative spectra conditions: scan speed, 150 nm min^{-1}; spectral slit-width, 2 nm; $\Delta\lambda$ (derivative algorithm bandwidth), 10 nm.

Standard solution and calibration curves for mass fragmentography

The standard solution used for mass fragmentography was a chloroformic solution containing phenylbutazone (1 mg ml^{-1}), II (0.01 mg ml^{-1}), III (0.01 mg ml^{-1}), IV (0.01 mg ml^{-1}) and phenazone (0.01 mg ml^{-1}) as internal standard. The samples were introduced into the mass spectrometer by a direct inlet system *via* a probe, by evaporating the solvent under reduced pressure prior to the probe introduction.

The fragmentographic analysis was achieved by the single-ion monitoring technique, using the following ions: m/z = 324 for compound II (M); m/z = 282 for compounds III ($M-CO_2$) and V (M); m/z = 298 for compound IV ($M-CO_2$) and m/z = 188 for phenazone (M). The magnet was focussed using m/z = 254 (octadecane).

Calibration curves were constructed by introducing different volumes (from 1 to 10 μl) of the standard solution previously described, and plotting the ratios of the areas of the monitored ions to that of the internal standard, against the quantities introduced

into the spectrometer. The amounts examined ranged from 10 to 100 ng, over which range the curves were linear for each compound examined.

Standard solutions and calibration curves for derivative UV-spectrophotometry

Standard solutions were prepared in ethanol containing: phenylbutazone (10^{-4}M) and one each of the decomposition products in turn (II, III, IV or V) in the concentration range $0.1–2.10^{-6}$M.

In the work of Fasanmade and Fell [13], a convention is proposed to represent the derivative amplitude (usually measured in arbitrary units). This convention is used here: the leading superscript denotes the order of derivative; the subscripts denote the positive and negative peaks or features measured, respectively.

The determination of these products was carried out, in the concentration range examined, by utilizing the peak–trough amplitude between 250 and 265 nm for II ($^3D_{250,265}$; h_1), and between 220 and 250 nm for III or IV in the third derivative spectrum ($^3D_{250,220}$; h_2), and the peak–trough amplitude between 257 and 275 nm in the second derivative spectrum for V ($^2D_{257,275}$; h_3). The following equations were obtained by regression analysis of data for the standard solutions previously described:

(1) $h_1 = 10.5 \times 10^6 x + 75.5$ (correlation coefficient, $r = 0.9918$, $n = 5$), where h_1 = peak–trough amplitude between 250 and 265 nm in the third derivative spectrum, and x = concentration of II (M)

(2) $h_2 = 12.5 \times 10^6 x + 172.1$ ($r = 0.9908$, $n = 5$), where h_2 = peak–trough amplitude between 220 and 250 nm in the third derivative spectrum, and x = III (or IV) or III + IV concentration (M)

(3) $h_3 = 2.6 \times 10^6 x + 48.5$ ($r = 0.9927$, $n = 5$) where h_3 = peak–trough amplitude between 257 and 275 nm in the second derivative spectrum, and x = concentration of V (M).

All the peak–trough amplitudes were measured in mm on the scale ±1 absorbance units.

Sample analysis by mass fragmentography

250 mg of bulk material were dissolved in 10 ml of chloroform containing 0.1 ml of a chloroformic solution of phenazone (1 mg ml^{-1}) as internal standard (final concentration: 0.01 mg ml^{-1}). As previously described, 2 µl of this solution were introduced *via* the probe into the spectrometer. The determination of the compounds II, III with V and IV was achieved using calibration curves generated *in situ*.

Sample analysis by derivative UV-spectrophotometry

The powdered bulk material was dissolved in ethanol in such a way as to obtain a 10^{-4}M solution of I. After filtration, the solution was analysed by derivative UV-spectrophotometry, using the equations previously reported. Concordance with these calibration data was checked by reference to single-point standards on a daily basis.

Results and Discussion

Mass fragmentography

The calibration curves were linear over the range examined, the correlation coefficients being: 0.998 for II and III and 0.996 for IV. The minimum concentration of impurities detectable by this method was 100 ppm.

On account of the presence of the fragment ion at m/z = 324 occurring in the mass spectrum of IV, in order to determine compound II using this ion it was necessary to subtract the contribution due to IV, calculated on the basis of the fragment ion at m/z = 298.

By this method it was not found possible to achieve the separate determination of III in the presence of V, and *vice versa*. The fragment ion m/z 282 could be used to determine the total of (III + V) in a sample of phenylbutazone.

The method was tested using samples prepared by adding known amounts of II, III and IV to phenylbutazone, purified by several recrystallizations. The results shown in Table 1 are the average of five determinations for each sample. The relative standard deviation of these results was *ca* 5%.

Table 2 reports the results obtained for the analysis of two commercial samples of phenylbutazone (bulk material). In these determinations the relative standard deviation was also found to be *ca* 5%.

Table 1
Mean results* obtained by mass fragmentography in control analyses of samples, prepared by adding known amounts of II, III and IV to pure phenylbutazone

Sample	II added (ppm)	II found (ppm)	Recovery (%)	III added (ppm)	III found (ppm)	Recovery (%)	IV added (ppm)	IV found (ppm)	Recovery (%)
1	2000	1900	95	1500	1400	93	1000	1000	100
2	1000	900	90	2000	1800	90	1500	1500	100
3	500	600	120	1000	1000	100	1000	800	80
4	1000	900	90	500	400	80	500	500	100
5	800	700	88	400	300	75	600	400	67

*n = 5 for each sample.

Table 2
Mean results* obtained in the analysis of commercial samples of phenylbutazone (bulk material) by mass fragmentography and derivative spectrophotometry

Sample	II found by MF (ppm)	II found by DS (ppm)	(III + V) found by MF† (ppm)	(III + IV) found by DS (ppm)	IV found by MF (ppm)	V found by DS (ppm)
1	ND	ND	2600	2500	800	1000
2	ND	ND	1100	1500	400	ND

ND = none detected.
*n = 5 for each sample.
†Using fragment ion m/z 282.
MF = mass fragmentography.
DS = derivative spectrophotometry.

Derivative UV-spectrophotometry

In Fig. 1 are shown the derivative spectra utilized for the determination of the phenylbutazone decomposition products. By this method it was not possible to achieve the separate determination of III and IV because of the similarity of their ultraviolet spectra. The theoretical background and practical implications of using the third-order derivative spectrum for quantitative analysis have been previously discussed by Fasanmade and Fell [13].

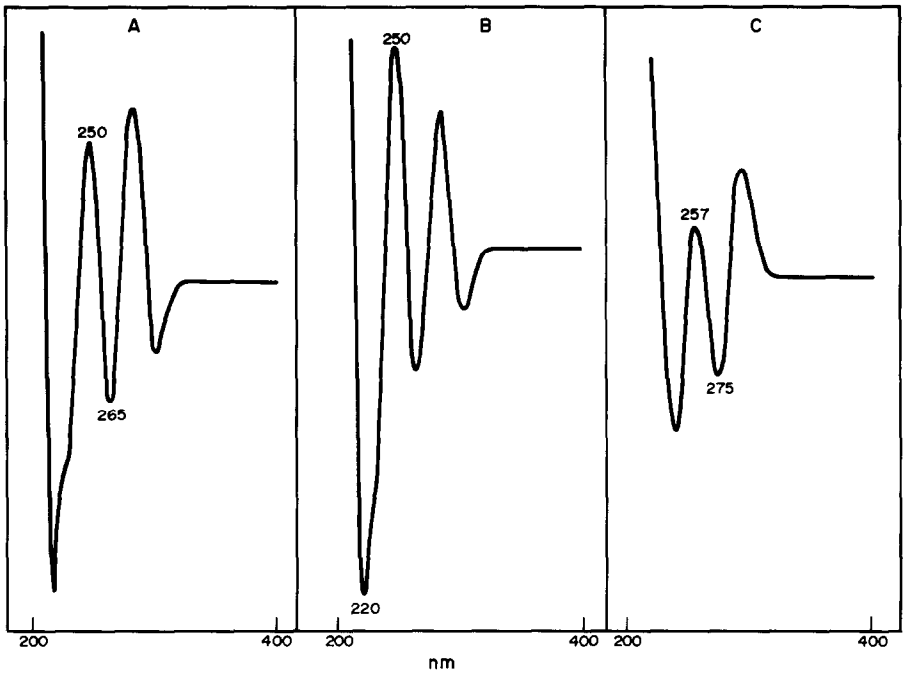


Figure 1
A: Third derivative ultraviolet spectrum of a 10^{-4}M ethanolic solution of phenylbutazone containing II (10^{-6}M). B: Third derivative ultraviolet spectrum of a 10^{-4}M ethanolic solution of phenylbutazone containing III (10^{-6}M). C: Second derivative ultraviolet spectrum of a 10^{-4}M ethanolic solution of phenylbutazone containing V (10^{-6}M).

The minimum concentration of II, III, IV and V detectable by derivative spectrophotometry was 1000 ppm. The determination of one of the decomposition products was not affected by the presence of the others.

In Table 2 are reported the results obtained for the analysis of the two samples of commercial phenylbutazone, previously analysed by mass fragmentography. The relative standard deviation observed with this technique was *ca* 2%.

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